

SUPPLEMENTAL PRELIMINARY AMENDMENT  
U.S. Appln. No. 09/915,543

least 70% sequence identity, more preferably at least 80% sequence identity, even more preferably at least 90% sequence identity, yet even more preferably at least 98% sequence identity, and most preferably 100% identity to (a) a nucleic acid molecule encoding a Lgs polypeptide having the sequence of amino acid residues from 1 to 1484 of Figure 2 (SEQ ID NO:1), or (b) the complement of the nucleic acid molecule of (a). --

Page 5, lines 1-14, delete in their entirety, and insert therefor

-- In another embodiment, the isolated nucleic acid containing a sequence having at least 30% sequence identity, preferably 50% sequence identity, more preferably at least 70% sequence identity, even more preferably 90% sequence identity, yet even more preferably 95% sequence identity to (a) a nucleic acid molecule encoding a human Lgs polypeptide of figure 10A (SEQ ID NO:16) or (b) the complement of the nucleic acid molecule of (a).

In a further embodiment, the isolated nucleic acid comprises a sequence with a low overall sequence identity but shows a sequence identity of at least 30%, preferably at least 50%, more preferably at least 70%, even more preferably at least 90% and most preferably 100% in the evolutionary conserved domains described in Figure 7B (SEQ ID NOs:2-13). --

Page 5, lines 17-24, delete in their entirety, and insert therefor

-- In another embodiment, the invention relates to a fragment of the *Drosophila* or human lgs nucleic acid sequences that can find use as hybridization probe. Such nucleic acid fragments

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

are about 18 to about 100 nucleotides in length, preferably from about 20 to about 60 nucleotides in length, most preferably from 20 to 50 nucleotides in length and can be derived from the nucleotides sequences shown in Figure 2 (SEQ ID NO:1) and Figure 10A (SEQ ID NO:16). --

Page 6, lines 1-12, delete in their entirety, and insert therefor

-- Methods of production, isolation and purification of the Lgs proteins, derivatives and analogs, e.g. by recombinant means, are also provided. In a specific embodiment, the invention concerns an isolated Lgs polypeptide or a fragment thereof, comprising an amino acid sequence of at least 80%, preferably at least about 85% sequence identity, more preferably at least 90% sequence identity, even more preferably at least 95% sequence identity, yet most preferably 100% identity with the sequence of amino acid residues 1 to 1464 of *Drosophila* Lgs of Figure 2 (SEQ ID NO:1) or amino acids residues of hLgs-1 of Figure 10B (SEQ ID NO:17). --

Page 6, lines 21-26, delete in their entirety, and insert therefor

-- A further aspect of the invention concerns an isolated full length Lgs polypeptide, comprising the sequence of amino acid residues 1 to 1464 of Figure 2 (SEQ ID NO:1), or any Lgs polypeptide or fragment thereof comprised in this invention sufficient to provide a binding site for an anti-Lgs antibody. --

SUPPLEMENTAL PRELIMINARY AMENDMENT  
U.S. Appln. No. 09/915,543

Page 8, lines 25-32, delete in their entirety, and insert therefor

-- In yet another embodiment the oligonucleotide is a double stranded lgs RNA molecule. Such ribonucleic acid fragments are about 18 to about 1000 nucleotides in length, preferably from about 20 to about 500 nucleotides in length, more preferably from 20 to 50, most preferably from 20 to 22 nucleotides in length and can be derived from the nucleotides sequences shown in Figure 2 (SEQ ID NO:1), 8A (SEQ ID NO:14) or 10A (SEQ ID NO:16). --

Page 9, lines 14-20, delete in their entirety, and insert therefor

-- The invention also relates to nucleotide sequences and the respective peptides derived thereof comprising at least one of the homology domains between Drosophila and human Lgs described in Figure 7B (SEQ ID NOS:2-13) and the use of said peptides to block Lgs function in cancer cells. Furthermore, the present invention comprises specific compounds that bind to said domains. --

Page 10, lines 17-22, delete in their entirety, and insert therefor

-- **Figure 2** The Drosophila lgs sequence (SEQ ID NO:1). cDNA is shown with introns from flies genomic DNA, introns are underlined. The first in-frame stop codon upstream of the ORF is underlined, the Kozak/Cavener sequence upstream of the initiator codon is marked by a bold underline, the beginning of the poly(A) tail is italicised. --

SUPPLEMENTAL PRELIMINARY AMENDMENT  
U.S. Appln. No. 09/915,543

Page 14, lines 15-28, delete in their entirety, and insert therefor

-- **Figure 7 (A)** Distribution of short local alignments (sequence homology domains) between dLgs and hLgs/Bcl9. The number of each alignment refers to figure 7B (SEQ ID NOs:2-13) which displays them in detail. A similar degree of homology is obtained by comparing homologues domains of dLgs and the predicted amino acid sequence of hLgs-1. hLgs/Bcl9 and hLgs display up to 95% homology in the same domains,

(B) Local alignments of dLgs with hLgs/Bcl9 (SEQ ID NOs:2-13). A WWW server implementation of LALIGN (version 2.0u63 was used (matrix: paml20; gap penalties: -14/-4; alignment 4 edited by hand)).

**Figure 8** The human lgs/bcl9 sequence.

(A) cDNA sequence (SEQ ID NO:14).

(B) Protein sequence (SEQ ID NO:15). --

Page 14, line 29-35, delete in their entirety.

Page 15, lines 1-26, delete in their entirety, and insert therefor

-- **Figure 9** Prediction of the formation of coiled-coil structures by wild type dLgs, 4 mutant dLgs forms, and hLgs/Bcl9. One occurrence of a coiled coil between amino acids 526-539 is predicted for dLgs, and the overall picture is somewhat similar for hLgs/Bcl9. The peak is lost in dLGS<sup>17E</sup> with the single amino acids exchange at position 531, and it is cut off by premature termination in the case of dLGS<sup>20F</sup>. dLGS<sup>17P</sup> with an amino acids exchange at position 532 has a reduced score, and

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

the homozygous viable allele *dlgs*<sup>21L</sup> with an amino acid exchange at position 509 is unaffected. A WWW server implementation of COILS version 2.1 was used with the MTDK matrix and without weights (Lupas, Van Dyke et al. 1991; Lupas 1997). All major peaks represent results obtained with a 14-residue window, the main peak also scores weakly with a 21-residue window, but nothing is detected with a 28-residue window. Remarkably, these mutated amino acids disrupting dLgs function are conserved in hLgs/Bcl9 (Figures 7A-7B (SEQ ID NOS:2-13)).

**Figure 10 (A)** Putative *hLgs/bcl9* homologue (*hlgs-1*) partial C-terminal cDNA (SEQ ID NO:16). Found by Blast search against hLgs/Bcl9 protein sequence. Following *hs\_genome/GS\_mRNA* was found which contains part of the *hlgs-1* cDNA sequence: *lcl|Hs11\_9491\_24\_72\_2*. Most of the N-terminal region can be derived e.g. from following EST: BF752124, D63746, BG116685, and the *hs\_genome/GS\_mRNA*: *lcl|Hs11\_9491\_22\_28\_8* (amino acid 1-225) (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>),

**(B)** Predicted protein (C-terminal part of *hlgs-1*) (fragment) (SEQ ID NO:17) derived by translation of the predicted cDNA in Figure 10(A) (SEQ ID NO:16). The N-terminus can to be derived by translation of the EST described above. The proteins contain all lgs sequence homology domains described in Figures 7A-7B (SEQ ID NOS:2-13). --

Page 16, delete in its entirety.

Page 17, delete in its entirety.

Page 18, delete in its entirety.

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

Page 19, lines 1-35, delete in their entirety, and insert therefor

-- **Figure 12** *In Vitro* Binding Assays, fine mapping. Proteins were *in vitro* translated (IVT) using reticulocyte lysates (TNT-lysates, Promega Corporation) containing [<sup>35</sup>S]-methionine (Amersham Pharmacia Biotech). Glutathione S-transferase (GST) fusion proteins were immobilized on glutathione-Sepharose and blocked in binding buffer (20 mM Tris pH 8.0, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 10 % glycerol, 0.5% NP40, 0.05% BSA, and proteinase inhibitors) for 45 min. Two µg of immobilized GST proteins were then incubated for 1.5 hrs with 0.5-4 µl of IVT proteins in binding buffer. The beads were washed four times in washing buffer (20 mM Tris pH 8.0, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 0.5% NP40) and boiled in Laemmli SDS sample buffer. The use of equivalent amounts of intact GST fusion proteins and successful IVT was confirmed by SDS-PAGE analysis using Coomassie staining or autoradiography, respectively. **(A)** Binding of IVT dLgs fragments to GST-Arm (top), and of IVT ΔArm to GST-dLgs-fragments (bottom). **(B)** Precise mapping of the Arm binding sites in dLgs (top), and of the β-Cat binding sites in hLgs (bottom). The figures depict the binding of *in vitro* translated dLgs and hLgs fragments to GST-Arm and GST-β-Cat, respectively. The minimal protein fragment, which still binds to Arm or β-Cat comprises the dLgs-hLgs sequence homology domain 2 of Figures 7A-7B (SEQ ID NOs:4-5). **(C)** Precise mapping of the Lgs binding sites in Arm. *In vitro* translated Arm fragment were tested for their binding to GST-dLgs(1-732).

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

**Figure 13** Binding of mutants dLgs and hLgs to Arm/ $\beta$ -Cat.

(A) Co-immunoprecipitation of mutant HA-dLgs-17E protein with GFP fused-Arm, -dTip, -dAPC and -Shaggy. HEK293 cells at 50% confluence were transfected by a lipofection method. Seven  $\mu$ g of DNA were diluted into 0.8 ml of OPTI-MEM Medium (Life Technologies, Inc.) and combined with 20  $\mu$ l of Lipofectamine (Life Technologies, Inc.) in 0.8 ml OPTI-MEM. After incubation for 20 min, 1.6 ml of OPTI-MEM was added and the mixtures were overlaid onto monolayers of cells. After culturing at 37°C/5% CO<sub>2</sub> for 6 hr, 3 ml of OPTI-MEM containing 20% fetal calf serum (FCS) was added to the cultures. Cells were lysed 25 h after transfection in co-IP buffer (20 mM Tris HCl pH 7.5, 140 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 1 mM EDTA, 1 mM DTT, 1% Triton-X100, 10% glycerol, 1 mM Natrium vanadate, 50 mM NaF, and protease inhibitors). Immunoprecipitations were performed in co-IP buffer either using the rat IgG<sub>1</sub> anti-HA monoclonal antibody or the mouse anti-myc monoclonal antibody (Clone 9E10, Calbiochem) conjugated to protein G-agarose (Boehringer Mannheim). Control Immunoprecipitations were performed using rat or mouse IgG (Sigma-Aldrich). After 3h incubation at 4°C, beads were washed 4 times in washing buffer (20 mM Tris HCl pH 7.5, 140 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 1 mM EDTA, 1 mM DTT, 1% Triton-X100, 1 mM Natrium vanadate, 50 mM NaF) and resuspended in 25  $\mu$ l of Laemmli buffer. Immune complexes were analyzed by SDS-PAGE/immunoblot assay using anti-GFP monoclonal antibody (Clontech Laboratories Inc.), followed by horseradish peroxidase conjugated secondary antibody (Amersham Pharmacia Biotech). Detection was performed using an

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

enhanced chemiluminescence detection method (ECL, Amersham Pharmacia Biotech). (B-C) In Vitro Binding Assays. Proteins were *in vitro* translated (IVT) using reticulocyte lysates (TNT-lysates, Promega Corporation) containing [<sup>35</sup>S]-methionine. Glutathione S-transferase (GST) fusion proteins were immobilized on glutathione-Sepharose and blocked in binding buffer (20 mM Tris pH 8.0, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 10% glycerol, 0.5% NP40, 0.05% BSA, and proteinase inhibitors) for 45 min. Two µg of immobilized GST proteins were then incubated for 1.5 hrs with 0.5-4 µl of IVT proteins in binding buffer. The beads were washed four times in washing buffer (20 mM Tris pH 8.0, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 0.5% NP40) and boiled in Laemmli SDS sample buffer. The use of equivalent amounts of intact GST fusion proteins and successful IVT was confirmed by SDS-PAGE analysis using Coomassie staining or autoradiography, respectively. (B) Binding of IVT wild type dLgs and dLgs-17E mutant to GST-ΔArm or GST alone (left panel), and of IVT wild type dLgs(354-555) or dLgs(354-555)-17E and -17P mutants to GST-ΔArm or GST alone (right panel). (C) Binding of IVT wild type hLgs/Bcl9 to GST alone or GST-β-Cat (top) and of IVT hLgs(Δ345-385) (also named hLgsdn) to GST alone or GST-β-Cat (bottom). Mutations in the conserved amino acids of the sequence homology domain 2 of Figures 7A-7B (SEQ ID NOs:4-5) abolish binding of Lgs to Arm and β-Cat.

**Figure 14** Down-regulation of dLgs protein levels by RNA interference. dLgs dsRNA was synthesized by PCR from pBS-dLgs (full length cDNA) using the T7 promoter containing dsRNA-Lgs-R1



**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

(TAATACGACTCACTATAGGGAGACCACTTCCATGCTCATTTCGTCATTA (SEQ ID  
NO:18)) and dsRNA-Lgs-F1  
(TAATACGACTCACTATAGGGAGACCACTAGGATCTCTCGACAACAATG (SEQ ID  
NO:19)) primers. As a control a PCR fragment was amplified from  
Arm cDNA using following primers: F primer  
(TAATACGACTCACTATAGGGAGACCACACAAGACCAAGTGGACGATATG (SEQ ID  
NO:20)),  
R Primer (TAATACGACTCACTATAGGGAGACCACAATTTGCAAGCAATCTGTGAC (SEQ  
ID NO:21)).

The amplified 700 base pairs products were purified using the  
PCR-Purification kit from Quiagen and the DNA was eluted with 50  
µl water. The DNA concentration was determined by UV absorbtion.  
The RNA synthesis reaction was then performed in 50µl volume  
with 1µg of the purified PCR products using the MEGAscript™ kits  
from Ambion. The DNA templates were removed with RNase-free  
DNAase and the dsRNAs were purified by phenol-chloroform  
extraction and ethanol precipitation. The RNAs became double-  
stranded during the synthesis reaction as confirmed by native  
agarose gel electrophoresis in TBE. For the RNA interference  
experiments, S2 cells were propagated in Schneider S2 Drosophila  
medium (GIBCO) supplemented with 10% FCS. One day before  
transfection one million cells were seeded into 6 well plates  
and growth overnight at 25°C. A total of 5 ug DNA and dsRNA was  
complexed with 20 ul of CellFectine lipid mix (GIBCO) in 1.2 ml  
serum free growth medium (DES expression medium, Invitrogen,  
Carlsbad, USA). As a control, EGFP (Clontech Laboratories Inc.,  
Palo Alto, USA) protein was expressed in the same cells under  
the control of the methallothionin promoter (vector used: pMT-

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

V5/HISB, Invitrogen). The complexes were incubated for 15 minutes at RT and then added to the cells from which the normal growth medium was replaced with 1 ml serum free medium. Four hour later 1.2 ml growth medium supplemented with 30%FCS was added to the cells. One day after transfection the medium was replaced with fresh medium with 10% FCS. Where an expression plasmid under the control of the insect metallothionin promoter (pMT/V5-HisB, Invitrogen) was transfected together with the dsRNA, copper sulfate was added to the cells to a final concentration of 0.5 mM. Cells were lysed in RIPA buffer 2 days after transfection. The cleared lysates were analyzed by SDS-PAGE/immunoblot assay using anti-Lgs polyclonal antiserum described herein and anti-GFP monoclonal antibody (Clontech Laboratories Inc.), followed by horseradish peroxidase conjugated secondary antibody (Amersham Pharmacia Biotech). Detection was performed using an enhanced chemiluminescence detection method (ECL, Amersham Pharmacia Biotech). **Top panel** Downregulation of endogeneous Lgs expression by Lgs dsRNA. As a control, cells were treated with Arm dsRNA. EGFP expression is not affected by the treatment with lgs dsRNA. **Lower panel** Downregulation of exogeneous dLgs expression. dLgs levels are brought under endogeneous levels by Lgs dsRNA treatment. --

Page 23, lines 3-30, delete in their entirety, and insert therefor

-- dLgs is located on the fourth chromosome. The dlgs gene was cloned by positional cloning and genomic walk, techniques frequently used by persons skilled in the art. Dlgs encodes for a 1464 amino acid protein of an expected molecular mass of 153

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

kDa. The dLgs protein is predicted to be predominantly hydrophilic and positively charged with a small hydrophobic stretch around amino acid 300 (Figure 2 (SEQ ID NO:1)). Neither obvious dLgs homologue nor any characterized functional motif can be found by common search tools (<http://dot.imgen.bcm.tmc.edu:9331>). However, by modification of the standard search parameters, several short stretches of amino acids within the dLgs protein are found to be highly homologue to a human protein, known as Bcl9, which has been linked to the development of B-cell lymphoma (Willis, Zalcborg et al. 1998; Busson-Le Coniat, Salomon-Nguyen et al. 1999), and to several translated EST coming from a predicted gene on chromosom 11. Interestingly, Bcl9, from now on named hLgs/Bcl9, displays similar structural feature compared to dLgs, like length, hydrophility and the presence of a predicted coiled region (Figure 9). In addition, it is remarkable that the short stretches of homology occur in a similar spacing and in the same succession as in dLgs (Figure 7A). As we show below, despite the overall very modest homology, hLgs/Bcl9 revealed to be the true functional human homologue of dLgs, and its function, as well as any lgs homologues, is hence part of the present invention. --

Page 24, lines 5-18, delete in their entirety, and insert therefor

-- The interaction with Arm is also confirmed in mammalian cells, where dLgs can be directed to the nucleus in the presence of nuclear but not cytoplasmic Arm (Figure 5A). Moreover, when co-transfected with Arm, Lgs increases the transcriptional

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

activity of hTcf (see examples and Figure 6). Similarly, we report herein the binding of hLgs/Bcl9 to  $\beta$ -Cat and its effect on  $\beta$ -Cat dependent transcriptional activation. We also demonstrate that dLgs and hLgs bind Arm and  $\beta$ -Cat, respectively, with the homology region No. 2 described in Figures 7A-7B (SEQ ID NOs:4-5), and that the homology region No. 1 is also essential for Lgs function (since it binds to Doll, another essential component of the Wg/Wnt pathway (provisional patent application No. 60/221,502)). --

Page 26, lines 1-19, delete in their entirety, and insert therefor

-- The invention also relates to Lgs nucleotide sequences and the respective peptides derived thereof comprising at least one of the homology domains between Drosophila and human Lgs described in Figures 7A-7B (SEQ ID NOs:2-13) and the use of said peptides to block Lgs function in cancer cells. Suitable techniques are known in the art for administering peptide to tumors. This can be achieved by direct administration of the peptide itself together with an appropriate pharmaceutical preparation which allow the penetration of such peptides into cells, or by mean of a gene therapy format. The latter bases of the administration of a DNA sequence coding for the peptide using suitable expression vectors. Such vectors are known in the art and it is in the skill of the artisan to select an appropriate one. In the tumor cells, the peptides will bind to their interaction partner, e.g.  $\beta$ -Cat if the homology domain 2 peptide is chosen, and titrate it away from the endogeneous Lgs

SUPPLEMENTAL PRELIMINARY AMENDMENT  
U.S. Appln. No. 09/915,543

proteins thus preventing expression of target genes by uncontrolled  $\beta$ -Cat. --

Page 26, lines 30-33, delete in their entirety.

Page 27, lines 1-15, delete in their entirety and insert therefor

-- A "wild type Lgs sequence" comprises a polypeptide having the same amino acid sequence as a Lgs protein derived from nature. Such wild type sequence of Lgs can be isolated from nature or produced by recombinant and/or synthetic means. The term "wild type sequence Lgs" specifically encompasses naturally occurring truncated forms, naturally occurring variant forms (e.g., alternatively spliced forms) and naturally occurring allelic variants of Lgs. In one embodiment of the invention, the native Lgs sequence is a mature or full-length Lgs sequence comprising amino acids 1 to 1464 of Figure 2 (SEQ ID NO:1) or amino acids 1 to 1394 of Figure 8B (SEQ ID NO:15).

"Lgs variant" means an active Lgs, having at least about 50% amino acid sequence identity with the amino acid sequence of residue 1 to 1464 of the *Drosophila* Lgs polypeptide of the sequence of Figure 2 (SEQ ID NO:1) or amino acids 1 to 1394 of Figure 8B (SEQ ID NO:15). The term "lgs variant" however, does also include functional homologues of Lgs in the Wnt pathway; --

Page 30, lines 10-16, delete in their entirety, and insert therefor

-- The following method describes use of a non-repetitive nucleotide sequence of *lgs* as a hybridization probe. The method can be applied to screen for *lgs* homologues as well. DNA

SUPPLEMENTAL PRELIMINARY AMENDMENT  
U.S. Appln. No. 09/915,543

A-17  
cont comprising the sequence of *lgs* (as shown in Figures 2 (SEQ ID NO:2), 8A (SEQ ID NO:14) and 10A (SEQ ID NO:16)) is employed as probe to screen for homologue DNAs (such as those included in cDNA or genomic libraries). --

Page 39, lines 18-38, delete in their entirety.

Page 40, lines 1-14, delete in their entirety, and insert therefor

A-18 -- *Lgs* dsRNA can be made from cDNA or genomic DNA templates, as long as most of the dsRNA corresponds to exon regions. Normally, target regions of 700 to 800 base pair are the most active. However, it is known that dsRNAs as short as 200 base pair and as long as 2000 base pairs have potent interfering activities. Both RNA strands can be synthesized simultaneously from a PCR fragment, which contains for instance a T7, SP6 or a T3 promoter on each end. This PCR fragment can be generated by amplification of *Lgs* cDNA or genomic DNA with 2 primers containing e.g. T7-polymerase binding sites. Primers complementary sequences should be 20 to 24 nucleotides in length with a 22 nucleotides optimum and 60°C optimum T<sub>m</sub>. The 5' end of each primer should correspond to e.g. a 27 nucleotides T7 promoter sequence (TAATACGACTCACTATAGGGAGACCAC (SEQ ID NO:22)). The PCR reaction is then performed with a suitable template containing *Lgs* sequences. Taq polymerase gives the best yields, but another polymerase like Pfu may be used, too. The first 10 cycles should have a 40°C annealing step, followed by 35 cycles with a 55°C annealing step. DMSO can be added to a final concentration of 5% when needed. Phenol-chloroform extract and ethanol precipitation in NH<sub>4</sub>OAc may be used to isolate the PCR

SUPPLEMENTAL PRELIMINARY AMENDMENT  
U.S. Appln. No. 09/915,543

A18  
cont

template from the reaction mix however other commercially available PCR-purification kit can be used as well. The RNA synthesis reaction can be performed in 50  $\mu$ l volume with 1  $\mu$ g of PCR DNA template using an appropriate RNA polymerase. The MEGAscript™ kits from Ambion work very well. The RNA becomes double-stranded during the synthesis reaction. The DNA template can be removed with RNase-free DNAase and the dsRNA can be purified by phenol-chloroform extraction and ethanol precipitation. Typical yields of RNA from 1  $\mu$ g DNA template are in the 80 to 120  $\mu$ g range. dsRNA is stored as a NaOAc/ethanol precipitate at -80°C until immediately before use. --

Page 41, lines 6-32, delete in their entirety, and insert therefor

A19

-- Bcl9, a human protein involved in B-cell lymphoma was identified by searching a public sequence database (<http://www.ch.embnet.org/software/aBLAST.html>) with fragments of about 500 amino acids of the Drosophila Lgs protein. The matrix used was Pam70 and the parameters were set so that repetitive sequences were filtered out. Although the overall homology of Bcl9 and dLgs is very low, they share several short stretches of amino acids with high homology and in the same sequential order (see Figures 7A-7B (SEQ ID NOS:2-13)). Local alignments were generated using A WWW server implementation of LALIGN (version 2.0u6319919. The parameters used are: matrix: pam120; gap penalties: -14/-4; alignment 4 edited by hand. The hlgs-1 gene was found by searching the public high throughput sequence database for predicted coding sequences (cDNA) with homology to the translated sequence of Bcl9 protein

**SUPPLEMENTAL PRELIMINARY AMENDMENT**  
**U.S. Appln. No. 09/915,543**

Al9  
cont

fragments (Figures 10A (SEQ ID NO:16)-10B (SEQ ID NO:17)). The program used was tblastn, whereas the parameters and matrixes were the same as described above for Lgs. The gene is situated on chromosome 11 and several EST are present in the public human genome databases. Transalation of the predicted cDNA and EST and a first assembly attempt results in a predicted protein containing all the homology domains of Figures 7A-7B (SEQ ID NOs:2-13). For instance hLgs-1 has a 54% and 57% amino acids identity with dLgs and hLgs, respectively, in domain 1, and a 23% and 60% amino acid identity, respectively, in domain 2 (data not shown). --

Page 43, lines 30-34, delete in their entirety.

Page 44, lines 1-18, delete in their entirety, and insert therefor

A20

-- To demonstrate the essential role of the sequence homology domains (HD) of Lgs described in Figures 7A-7B (SEQ ID NOs:2-13) for the propagation of the Wnt signaling pathway, a Tcf-reporter gene assay was performed. In this, HEK293 cells at 50% confluence were plated into 24-well plates and transfected by a lipofection method. 240 ng of TOPFLASH luciferase reporter plasmid (Upstate biotechnology, New York, USA), 4 ng of pcDNA3- $\Delta$ Arm, 200 ng of pcDNA3-EGFP-hLgs-peptide and 10 ng of a renilla luciferase reporter plasmid pRL-SV40 (Promega Corporation, Madison USA) were diluted into 25  $\mu$ l of OPTI-MEM Medium (Life Technologies, Inc.) and combined with 1.2  $\mu$ l of Lipofectamine (Life Technologies, Inc.) in 25  $\mu$ l OPTI-MEM. After incubation for 20 min, 0.175 ml of OPTI-MEM was added and the mixtures were overlaid onto monolayers of cells. After culturing at 37°C/5%